# Genetics and genomic basis of sleep in healthy humans

Landolt, Hans-Peter ; Dijk, D J

Abstract: Chapter Highlights * Distinct characteristics of human sleep are regulated by different molecular and genetic mechanisms with different degrees of heritability. * The genetics of sleep and sleep-wake regulation are still relatively unknown when compared to the genetics of other complex traits. * The sleep EEG is one of the most heritable traits in humans. Elucidating the underlying genes may reveal novel sleep functions. * Independent replication and confirmation of most of currently proposed genetic associations with distinct characteristics of sleep and sleep regulatory processes in high-quality protocols and data sets is required.



Originally published at:
Landolt, Hans-Peter; Dijk, D J (2017). Genetics and genomic basis of sleep in healthy humans. In: Kryger, M H; Roth, T; Dement, WC. Principles and Practice in Sleep Medicine. Philadelphia: Elsevier, 310-321.

# GENETICS OF SLEEP IN HEALTHY HUMANS

*Hans-Peter Landolt [1,2] and Derk-Jan Dijk[3]*

[1] Institute of Pharmacology & Toxicology, University of Zürich, Zürich, Switzerland

[2] Zürich Center for interdisciplinary Sleep Research (ZiS), University of Zürich, Switzerland

[3] Surrey Sleep Research Centre, University of Surrey, Guildford, United Kingdom

Submitted to: ***Principles and Practice in Sleep Medicine***

**Addresses for correspondence:**

Hans-Peter Landolt, Ph.D.

Institute of Pharmacology & Toxicology

University of Zürich

Winterthurerstrasse 190

8057 Zürich

Switzerland

Tel. + 41 - 44 - 635 59 53

Fax. + 41 - 44 - 635 57 07

e-mail: landolt@pharma.uzh.ch

Derk-Jan Dijk, Ph.D.

Surrey Sleep Research Centre

University of Surrey

Egerton Road

Guildford, Surrey, GU2 7XP

UK

Tel. + 44 - 1483 - 68 93 41

Fax. + 44 - 870 - 137 15 90

e-mail: d.j.dijk@surrey.ac.uk

**Chapter Highlights**

- Distinct characteristics of human sleep are regulated by different molecular and genetic mechanisms with different degrees of heritability.
- The genetics of sleep and sleep-wake regulation are still relatively unknown when compared to the genetics of other complex traits.
- The sleep EEG is one of the most heritable traits in humans. Elucidating the underlying genes may reveal novel sleep functions.
- Independent replication and confirmation of most of currently proposed genetic associations with distinct characteristics of sleep and sleep regulatory processes in high-quality protocols and data sets is required.

**Evidence for trait-like and genotype-dependent differences in diurnal preference, sleep timing, sleep EEG, sleep architecture, and sleep duration**

Many aspects of sleep and sleep-wake regulation are highly variable among individuals, yet highly stable within individuals. Uncovering genetic factors contributing to these trait-like individual differences in healthy humans constitutes one of the most promising avenues to foster our understanding of the neurobiology of sleep in health and disease. This chapter will summarize the current evidence for genotype-dependent differences in timing, duration and structure of sleep, as well as the sleep EEG in healthy individuals. Table 1 summarizes known variations in genes and their functional significance that were investigated to date whether they contribute to genotype-dependent differences in diurnal preference or sleep timing, sleep EEG sleep, sleep structure and duration. We will also review how these differences may relate to the homeostatic and circadian regulation of sleep. Several sleep characteristics differ between the sexes and ethnic groups, but these differences will not be discussed here.

The manifestation and regulation of sleep and the sleep EEG reflect different aspects of complex behaviors. Each of these aspects is likely to be under the control of multiple genes, which may interact, and are also influenced by the environment and other factors such as age. In humans, little is currently known about the genes that contribute to the trait-like, individual “sleep-phenotypes”. Similarly, little is known about the genes that contribute to individual “circadian-phenotypes”, although a considerable number of genes that contribute to circadian rhythmicity have been discovered in animals.

Two main techniques for the genetic dissection of normal human sleep are currently available. The first is to examine the impact of candidate genes, for which evidence exists that they are implicated in sleep and sleep-wake regulation. With this method, individuals with distinct genotypes of known genetic polymorphisms are prospectively studied in the sleep laboratory. This approach precludes discovery of novel “sleep genes”, but may help to understand the consequences

of these polymorphism for sleep physiology. By contrast, genome-wide association (GWA) studies may lead to the identification of novel "sleep genes", which may lead to the discovery of novel sleep regulatory pathways. These studies, however, require very large sample sizes and multiple replications. The weaknesses and strengths of these strategies have been discussed in detail [1].

Large inter-individual differences are observed in preferred time of day for completion of distinct cognitive tasks, sleep timing, sleep EEG, sleep structure, and sleep duration. Genes contribute to each of these phenotypes, and a high degree of heritability, i.e., the percentage of variance explained by overall genetic effects, has been demonstrated for these variables. For some of these variables the magnitude of inter-individual differences exceeds by far the size of the effects of manipulations of sleep regulatory processes, such as sleep deprivation [2].

**Genes contributing to human morningness/eveningness and timing of sleep**

*Candidate genes*

The timing of the peaks and troughs of daytime alertness and the timing of nocturnal sleep (i.e., diurnal preference) are highly variable among healthy individuals [3]. Some of us go to sleep when others wake up. Self-rating scales such as the Horne-Östberg morningness-evenigness Questionnaire (MEQ) and the Diurnal Type Scale show normal distribution along an "eveningness – morningness" axis [4, 5], indicating the contribution of additive, small effects of multiple genes in combination with the environment. Recent studies in large numbers of monozygotic (MZ) and dizygotic (DZ) twin pairs and population- and family-based cohorts revealed roughly 50 % heritability for diurnal preference [6] and 22-25 % for habitual bedtime [7, 8].

Morningness/eveningness and timing of sleep are thought to be determined in part by characteristics of the central circadian oscillator, and associations between the intrinsic period and/ or phase marker of this oscillator and diurnal preference have been reported [9-12]. These oscillators consists at the molecular level of a network of inter-locked transcriptional/translational feedback

loops, which involve several clock-related genes including the transcription regulators *CLOCK*, *BMAL1*, *PER1-3, CRY1-2* and other genes. This knowledge has provided an obvious rational basis for the search for associations between these genes and morningness/eveningness and altered sleep timing.

The effect of a single nucleotide polymorphism (SNP) in the 3'-untranslated region (UTR) of the human "Circadian locomotor output cycles kaput" gene (*CLOCK*) located on chromosome 4 on diurnal preference was first studied in middle-aged adults. This SNP may affect stability and half-life of messenger RNA [13], and thus alter the protein level that is finally translated. Katzenberg *et al.* [14] reported that homozygous carriers of the 3111C allele have increased evening preference for mental activities and sleep, with delays ranging from 10 to 44 minutes when compared to individuals carrying the 3111T allele. A similar association with diurnal preference was found in a Japanese population, and MEQ scores were significantly correlated with sleep onset time and wake time [5]. By contrast, studies in healthy European and Brazilian samples failed to confirm an association between genetic variation in *CLOCK* and diurnal preference [15, 16]. Interestingly, an almost complete linkage disequilibrium was shown between the 3111T>C and the 257T>G polymorphism located in the other extremity of this gene [16]. Full-length analysis of secondary mRNA structure revealed no interaction between the two polymorphisms.

Mouse *Per1* and *Per2* are importantly involved in maintaining circadian rhythmicity [17], and possible associations between variation in these genes and diurnal preference were thus also investigated in humans. Screening for missense mutations and functional or synonymous polymorphisms in promoter, 5'- and 3'-UTR and coding regions of the period-1 gene (*PER1*) in volunteers with extreme diurnal preference and patients with delayed sleep phase syndrome (DSPS) remained initially unsuccessful [18, 19]. By contrast, the distribution of the C and T alleles of a silent polymorphism in exon 18 was found to differ between extreme morning and evening types [19]. Thus, the frequency of the 2434C allele was roughly double in subjects with extreme morning preference (24 %) compared to subjects with extreme evening preference (12 %). This polymorphism may be

linked to another functional polymorphism or directly affect *PER1* expression at the translational level [19]. In a candidate gene association study with replication, a polymorphism in *PER1* (single nucleotide polymorphism identification number: rs7221412) was found to be associated with sleep timing based on actigraphy [20].

A missense mutation in the human period-2 gene (*PER2*) currently provides the most striking example of a direct link with between genetic variation in a clock gene and changed circadian rhythms. Linkage analyses in families afflicted with familial advanced sleep phase syndrome (FASPS) revealed associations with functional polymorphisms of *PER2* that cause altered amino acid sequences in regions important for phosphorylation of this protein [21] and a mutation in Caseine Kinase Delta (CKδ), which plays an important role in phosphorylation [22]. The subsequent finding in a transgenic mouse model expressing the human FASPS mutation that casein kinase I delta (CKIδ) can regulate circadian period through PER2 provided further important evidence that this gene is importantly involved in the mechanisms of circadian rhythm regulation in humans [23]. In accordance with this notion, a C111G polymorphism located in the 5'-UTR of *PER2* modulates diurnal preference in healthy volunteers [24]. Thus, the 111G allele is significantly more prevalent in subjects with extreme morning preference (14 %) than in individuals with extreme evening preference (3 %). Computer simulation predicted that the 111G allele has different secondary RNA structure than the 111C allele and that the two transcripts may be differently translated [24].

Findings in mice suggest that Per3 has primarily functions outside the central circadian clock [17, 25]. Nevertheless, a variable-number-tandem-repeat (VNTR) polymorphism in the human period-3 gene (*PER3*) also appears to modulate morning and evening preference. A 54-nucleotide sequence located in a coding region of this gene on human chromosome 1 is either repeated in four or five units. This difference may alter the dynamics in PER3 protein phosphorylation. The longer 5-repeat allele was associated in European and Brazilian populations with morning preference, and the shorter 4-repeat allele with evening preference, respectively [26-28]. More recently in a sample of 925 healthy Japanese controls the *PER3* SNP rs228697, which is associated with a proline to alanine

amino acid substitution, was shown to be associated with diurnal preference such that the major C allele was more prevalent in morning types and the minor G allele more common in evening types [29]. In addition, in a sample of 966 young adults in Britain, a significant association between SNP rs10462020 of *PER3* and diurnal preference was reported such that G/G individuals had an increased morning preference compared to T/G and T/T individuals [30]. In this study an association between a polymorphism (rs922270) in *BMAL* (*ARNTL2*) and diurnal preference was also reported.

The gene encoding arylalkylamine *N*-acetyltransferase (*AANAT*) is located on human chromosome 17q25. This enzyme plays a key-role in melatonin synthesis and may, thus, be important for diurnal preference and circadian rhythm disturbances. Comparison in a Japanese population between 50 outpatients diagnosed with DSPS and 161 unrelated healthy controls suggested that the frequency of a seldomly occurring threonine allele at codon 129 is significantly higher in patients than in controls [31]. This association was not confirmed in a Brazilian population where virtually no allelic variation at this position was found [32]. In a small study conducted in Singapore, it was suggested that a commonly occurring, silent -263G>C polymorphism of *AANAT* modulates sleep timing and sleep duration (also see below) among healthy students [33].

*Genome-wide association (GWA) studies*

Only three GWA studies of sleep-related phenotypes are currently available in humans [7, 34, 35]. In the Framingham Heart Study 100K Project [7], phenotypic and genetic analyses were conducted in 749 subjects and revealed a heritability estimate for habitual bedtime of 22 %. This small study suggests that a non-synonymous polymorphism in a coding region of the gene encoding neuropeptide S receptor 1 (*NPSR1*) is a possible modulator of usual bedtime as obtained from a self-completion questionnaire. This polymorphism leads to a gain-of-function mutation in the receptor protein by increasing the sensitivity for neuropeptide S receptor 10-fold [36]. Although a possible association of NPSR1 to weekday bedtime is interesting, it has to be kept in mind that the statistical

power of this pilot study is limited and necessary replication of this finding in independent samples is lacking. A recent analysis of a larger sample of the Framingham Offspring Cohort did not parallel the prior result [8].

**The sleep EEG is among the most heritable traits in humans**

Visual sleep state scoring relies on arbitrarily defined criteria and can reveal only limited information about sleep physiology. To obtain more detailed insights, quantitative analyses of the EEG signal recorded during sleep have to be performed. A powerful approach to quantify amplitude and prevalence of EEG oscillations with distinct frequencies is power spectral analysis based on Fast-Fourier Transform (FFT) [37-39]. Recent studies strongly suggest that especially the sleep EEG, but also the waking EEG, are highly heritable traits in humans. All-night sleep EEG spectra derived from multiple recordings in healthy individuals show large inter-individual variation and high intra-individual stability [39, 40]. Buckelmüller *et al.* [40] recorded in 8 young men 2 pairs of baseline nights separated by 4 weeks. While the spectra in NREM sleep differed largely among the individuals, the absolute power values and the shape of each subject's spectra were impressively constant across all nights (Fig. 1). The largest differences among the subjects were present in the theta, alpha and sigma (~ 5-15 Hz) range. Hierarchical cluster analysis of Euclidean distances based on spectral values as feature vectors demonstrated that all 4 nights of each individual segregated into the same single cluster [40]. Similar results were obtained in REM sleep, and by other researchers in men and women of older age [39]. These data strongly suggest that the sleep EEG contains systematic and stable inter-individual differences, which are at least in part genetically determined.

This notion is further supported by two recent twin studies investigating the heritability of the sleep EEG. Ambrosius et al. [41] quantified the sleep EEG profiles in 35 pairs of MZ twins (17 male pairs, 18 female pairs; age range: 17-43 years) and 14 pairs of DZ twins (7 male pairs, 7 female pairs; age range: 18-26 years). Stable and robust inter-individual differences in a broad range of the NREM sleep EEG

were observed. Furthermore, intra-class correlation coefficients (ICC) of spectral power were significantly higher in MZ twins than in DZ twins [41]. The ICC reflect within-pair similarity of twin pairs. In frequencies between 0.75-13.75 Hz, the ICC equaled roughly 0.8 in MZ twins and roughly 0.6 in DZ twins. The differences between MC and DC twin pairs appeared most pronounced in theta and alpha (4.75-11.75 Hz) frequencies (see also [42]).

De Gennaro and colleagues [43] also conducted a twin study to test the hypothesis that the EEG in NREM sleep reflects a genetically-determined, individual "fingerprint". They recorded baseline and recovery sleep after sleep deprivation in 10 MZ and 10 DZ twin pairs (mean age: 24.6 ± 2.4 years; 5 male and 5 female pairs in each group) and observed highest variability in the 8-16 Hz range. In this frequency band, group similarity as quantified by an ICC procedure was more than double in MZ pairs (ICC = 0.934; 95 % confidence intervals: 0.911-0.965) than in DZ pairs (ICC = 0.459; 95 % confidence intervals: 0.371-0.546) (Fig. 2). This difference suggested 95.9 % heritability independently of sleep pressure [43]. As such, the sleep EEG qualifies as one of the most heritable traits known so far, only matched by heritability estimates for distinct brain characteristics like cortical grey matter distribution [1]. It may, thus, be likely that trait characteristics of rhythmic brain oscillations during sleep and distinct neuroanatomical features are inter-related.

In conclusion, accumulating evidence suggests that the sleep EEG is a highly heritable trait, yet the underlying genetic determinants are largely unknown. Nevertheless, more and more studies become available that investigated the effects of known allelic variants of candidate genes on the human sleep EEG (Table 1). The findings demonstrate that genetic variation of various cells, molecules and signaling pathways can profoundly modulate sleep EEG and other sleep phenotypes. Selected genes and pathways will be briefly discussed in the following paragraphs.

**Genes contributing to the sleep EEG**

*Circadian clock genes*

A wealth of studies in genetically modified mice and flies demonstrates that circadian clock genes are strong determinants of major characteristics of the sleep EEG [1, 44]. The only, yet intensively, studied "clock gene" variant in healthy humans is the above mentioned VNTR polymorphism of *PER3* (rs57875989) [45]. Apart from its impact on diurnal preference, this polymorphism also modulates the sleep EEG in NREM as well as in REM sleep. Compared to individuals with the *PER3*$^{4/4}$ genotype, young adult homozygous carriers of the long-repeat allele (*PER3*$^{5/5}$ genotype) exhibited higher EEG activity in the delta range (1-2 Hz) in NREM sleep and in the theta/alpha range (7-10 Hz) in REM sleep [46]. Partly similar observations were made in healthy older individuals between 55 and 75 years of age [47].

*Adenosinergic neuromodulation*

The neuromodulator adenosine is released in activity-dependent manner, and genes encoding adenosine-metabolizing enzymes and adenosine receptors are thought to play a major role in regulating the quality of sleep and wakefulness in animals and humans [1, 48]. Adenosine kinase and adenosine deaminase (ADA) importantly contribute to the regulation of extracellular adenosine levels [49]. Genetic studies in mice suggest that both enzymes are involved in sleep-wake homeostasis [50, 51]. In humans, the *ADA* gene is located on chromosome 20q13.11 and encodes two electrophoretic variants of ADA, referred to as ADA*1 and ADA*2 (rs73598374). The ADA*2 variant results from a guanine-to-adenine transition at nucleotide 22, which is translated into an asparagine-to-aspartic acid amino acid substitution at codon 8. The heterozygous ADA*1-2 (G/A) genotype shows reduced catalytic activity of ADA compared to homozygous individuals carrying the ADA*1 (G/G genotype) variant [52]. Rétey et al. [53] observed that this polymorphism affects the spectral composition of the sleep EEG. More specifically, EEG delta activity in NREM sleep (0.25-5.5 Hz) and REM sleep (2.0-2.25 and 3.5-4.75 Hz) was higher in the G/A genotype than in the G/G genotype [53]. Inspired by studies in inbred mice showing that the genomic region encoding *Ada* modifies the rate

at which sleep need accumulates during wakefulness [51], it was then examined whether individuals with G/A and G/G genotypes respond differently to sleep deprivation. In accordance with the original study, delta (0.75-1.5 Hz) activity in NREM sleep was elevated in the G/A genotype when compared to the G/G genotype in both baseline and recovery nights [54]. The *ADA* genotype-dependent EEG alterations, however, were not restricted to the low-delta range in NREM sleep, but also included a pronounced increase in theta/alpha frequencies (~ 6-12 Hz) in NREM sleep, REM sleep and wakefulness. Importantly, an independent study in a large epidemiological sample confirmed that A-allele carriers have higher delta power in NREM sleep and increased theta power in NREM and REM sleep when compared to homozygous G/G genotype carriers [55].

The effects of adenosine on target cells are mediated via four different subtypes of G-protein-coupled adenosine receptors: $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors. Physiological concentrations of endogenous adenosine can activate $A_1$, $A_{2A}$, and $A_3$ receptors. It is thought that adenosine modulates sleep primarily by binding to high-affinity $A_1$ and $A_{2A}$ receptors [48, 56]. No study yet investigated the possible effects of variants of the $A_1$ receptor gene on the human sleep EEG. By contrast, it was shown that the common variation rs5751876 of the adenosine $A_{2A}$ receptor gene (*ADORA2A*) located on chromosome 22q11.2 affects the EEG in NREM and REM sleep [53]. This polymorphism is linked to a 2592C>$T_{ins}$ polymorphism in the 3'-UTR of *ADORA2A* and may modulate receptor protein expression [57]. In a case-control study, Rétey and co-workers observed that EEG activity in the ~ 7-10 Hz range was invariably higher in all vigilance states in subjects with the C/C genotype of rs5751876 than in subjects with the T/T genotype [53]. Because the C-allele is thought to facilitate $A_{2A}$ receptor function when compared to the T-allele, these data may suggest that genetically increased $A_{2A}$ receptor-mediated signal transduction enhances EEG theta/alpha activity independently of sleep state.

*Neurotransmitters*

Accumulating evidence suggests a contribution of dopamine to sleep-wake regulation in

humans [58, 59]. The enzyme catechol-O-methyltransferase (COMT) plays a major role in the metabolic degradation of brain catecholamines, including dopamine. The gene encoding COMT is located on human chromosome 22q11.2, in proximity to *ADORA2A*. Human *COMT* contains a common functional 544G>A variation that alters the amino acid sequence of COMT protein at codon 158 from valine (Val) to methionine (Met) [60]. Individuals homozygous for the Val-allele show higher COMT activity and lower dopaminergic signaling in prefrontal cortex than Met/Met homozygotes [61, 62]. Sleep variables and the sleep EEG response did not differ between male carriers of Val/Val and Met/Met genotypes [63]. By contrast, the Val158Met polymorphism of *COMT* was associated with consistently lower EEG activity in the upper-alpha (11-13 Hz) range in in NREM sleep, REM sleep, and wakefulness in Val/Val compared to Met/Met homozygotes [64]. The difference in NREM sleep was present before and after sleep deprivation, and persisted after administration of the wake-promoting compound modafinil during prolonged wakefulness (Fig. 3). These data demonstrate that a functional variation of the *COMT* gene predicts robust inter-individual differences in the sleep EEG. In addition, this polymorphism profoundly affected the efficacy of modafinil to improve impaired well-being and cognitive functions after sleep deprivation [65]. Thus, two-time 100 mg modafinil potently improved vigor and well-being, and maintained baseline performance of executive functioning and vigilant attention throughout 40 hours prolonged wakefulness in 10 Val/Val homozygotes, yet the same dose was virtually ineffective in 12 Met/Met homozygotes. Interestingly, an opposite relationship between Val158Met genotype of *COMT* and measures of daytime sleepiness may be present in patients suffering from narcolepsy (see Clinical pearl).

*Signaling pathways*

Another functional polymorphism affecting the sleep EEG in theta/alpha frequencies is a guanine-to-adenine transition at nucleotide 196 of the gene encoding brain-derived neurotrophic factor (BDNF) (rs6265) [66, 67]. This polymorphism is located on human chromosome 11p13 and causes

a valine-to-methionine amino acid substitution at codon 66 of the pro-BDNF sequence. *In vitro* studies suggest that the presence of a Met-allele reduces intracellular trafficking and activity-dependent secretion of mature BDNF protein [68]. This polymorphism is typically associated with reduced performance on cognitive tasks that are also affected by sleep deprivation. Sleep and the sleep EEG were first investigated in case-control fashion in eleven carriers of the Val/Met genotype and eleven prospectively-matched Val/Val homozygotes. It was found that the Val66Met polymorphism of *BDNF* not only reduces response accuracy on a verbal 2-back working memory task but also modulated the spectral composition of the EEG in frequency- and vigilance state-specific manner [66]. More specifically, in baseline and recovery nights after sleep deprivation, delta, theta and low-alpha activity in NREM sleep EEG was lower in Met allele carriers than in Val/Val homozygotes. Importantly, the genotype-dependent differences in the theta/low-alpha band (~ 4-9 Hz) were recently confirmed in a large and ethnically diverse population-based epidemiological sample [67].

A point mutation at codon 178 (in rare cases also a mutation at codon 200) of the prion protein gene (*PRNP*) has been identified as the cause underlying the devastating disease, fatal familial insomnia (FFI) [69, 70]. Interestingly, while healthy relatives of FFI patients appear to have normal sleep EEG [71], the polymorphic codon 129 of the *PRNP* gene may influence EEG activity during sleep [72]. Subjects with Met/Val genotype showed lower slow-wave activity and higher spindle frequency activity than individuals with the Val/Val genotype, independent of codon 178.

**Genes contributing to sleep architecture**

Not only the sleep EEG, but also many variables characterizing sleep architecture demonstrate large variation among individuals and high stability within individuals [2, 39, 40, 73]. For example, the intra-class correlation coefficients, which estimate the intra-individual stability of a given variable across different conditions, i.e., baseline vs. sleep deprivation, was reported to be 0.73 for slow wave sleep (SWS) and 0.48 for REM sleep [2]. This observation suggests the presence of

trait-like, inter-individual differences in sleep physiology, which have a genetic basis. Indeed, twin studies show striking similarity and concordance in visually defined sleep variables in MZ twins, yet not in DZ twins. Already the first polysomnographic sleep studies in MZ twins revealed almost complete concordance in the temporal sequence of sleep stages [74]. Subsequent work showed that in particular those variables, which most reliably reflect sleep need are under tight genetic control. Apart from total sleep time, they include duration of NREM sleep stages, especially SWS, and density of rapid eye movements in REM sleep [75-77]. Linkowski [77] estimated that heritability of markers of sleep homeostasis is up to 90 % (REM density).

*Slow wave sleep*

A few studies have conducted polysomnographical assessment in defined genotypes. The *CLOCK* genotypes that were associated with diurnal preference [14], did not significantly affect sleep variables derived from nocturnal polysomnography. By contrast, it was found that young homozygous carriers of the long-repeat genotype of *PER3 (PER3$^{5/5}$)*, fell asleep more rapidly and showed more SWS particularly stage 4 sleep) when compared to homozygous 4-repeat individuals [46, 78]. A difference in SWS, yet on a lower level, was also observed in older people [47].

Similarly, with respect to polymorphism rs73598374 of *ADA*, healthy carriers of the ADA*2 allele (G/A genotype) showed significantly more SWS than subjects with the G/G genotype [53, 54]. All other sleep variables were similar in both *ADA* genotypes.

The impact of the Val66Met polymorphism of *BDNF* was also reflected in sleep architecture. In baseline and recovery nights, Val/Val allele carriers spent roughly 20 min more in deep stage 4 sleep than Val/Met allele carriers. By contrast, superficial stage 2 sleep was reduced [66] . Taken together, functional variation in the genes encoding *PER3*, *ADA* and *BDNF* not only modulate the spectral characteristics of the sleep EEG but also sleep architecture.

**Genes contributing to habitual sleep duration**

Habitual sleep duration shows large variation among healthy individuals, and the physiological sleep and circadian correlates of habitual short and long sleepers have been identified in small groups of subjects [79-81]. The temporal profiles of nocturnal melatonin and cortisol levels, body temperature and sleepiness under constant environmental conditions and in the absence of sleep suggest that the circadian pacemaker programs a longer biological night in long sleepers than in short sleepers [81]. Individual differences in this program may contribute to the large variation in habitual sleep duration, which shows a perfect normal distribution in the general population [82, 83]. Such a distribution is consistent with the influence of multiple, low-penetrance polymorphisms. Twin and GWA studies reported for sleep duration heritability estimates of 9-40 % [7, 35, 84-86].

*Circadian clock genes*

A candidate gene study of 194 SNPs in clock genes and self-reported sleep duration on the Munich ChronoType Questionnaire was recently conducted in a European population (n = 283) [87]. The top two associations were both located in the gene *CLOCK* on chromosome 4. With one of these variants, rs12649507, sleep duration was significantly associated in the original discovery sample, in a replication sample (n = 1'011), as well as in the meta-analysis of the two populations ($p < 0.009$) [87]. Two recent studies aimed at replicating this initial finding, yet revealed inconsistent results. While Evans et al. [88] reported successful replication of the previously described association in 2'527 male elderly participants, Lane et al. [89] found no evidence of an association. These authors collected objective polysomnographic data in three large independent cohorts of European ancestry. This analysis with > 99% power to detect an effect of similar magnitude as previously reported did not support a significant association of *CLOCK* variants with sleep duration.

Evidence for a role of clock genes in modulating sleep duration also came from work in a small family who apparently needed just 6 hours of sleep per night [90]. This family-based candidate

gene study revealed a point mutation in exon 5 of the gene encoding class E basic helix-loop-helix protein 41 (*BHLHE41*), also known as the transcriptional repressor gene *DEC2*. By this missense mutation (c.1151C>G), proline is replaced by arginine at amino acid position 384 (p.Pro384Arg) of BHLHE41 protein. This protein is part of the transcription factor family that is regulated by the mammalian circadian clock and influences the expression of *CLOCK/BMAL1* [91, 92]. Interestingly, knocking-in the human mutation into mice and *Drosophila* was reported to result in reduced sleep duration in transgenic animals [90]. Based on this study, other variants of the *BHLHE41* gene were searched for by DNA sequencing in two larger cohorts (n = 417) of healthy volunteers and two other rare variants in the same exon of *BHLHE41* were found [92]. The phenotypic data reported in three carriers of the non-synonomous variant c.1151C>A (p.Pro384Gln) and in one DZ twin pair discordant for the functional c.1086C>T (p.Tyr362His) polymorphism may suggest that variants which alter the suppression of *CLOCK/BMAL1* activation lead to short sleep, whereas a polymorphism that does not affect this suppression has no effect on sleep duration [92].

*Neurotransmitters*

It is well established that the regulation of sleep and mood are closely related. A regression analysis of 23 risk variants of major depressive disorder covering 12 different genes with self-reported sleep duration was conducted in 3'147 healthy individuals of two population-based Finnish cohorts. Polymorphism rs687577 (g.123445253A>C) of the gene *GRIA3* (ionotropic glutamate receptor, AMPA subunit 3) located on chromosome X was found to be significantly associated with sleep duration in healthy women [93]. More specifically, the frequency of the C/C genotype was highest in all age groups younger than 70 years in women reporting to sleep 8 hours or less. The frequency of this genotype decreased with longer sleep duration and individuals with 9-10 hour to sleep showed higher frequencies of C/A and A/A genotypes than mid-range sleepers (7-8 hours). It was concluded that mood disorders and short sleep may share a common genetic and biologic background involving glutamatergic

neurotransmission [93].

*Transporters*

It has long been suggested that serotonin (5-hydroxy-tryptamine, 5-HT) is critically involved in sleep-wake mechanisms [94], yet the specific roles for this neurotransmitter in sleep-wake regulation remain uncertain [95]. Current evidence supports the view that 5-HT contributes to the build-up of sleep need during wakefulness. Apart from its intra-cellular metabolism by monoamine oxidase, 5-HT is removed from the synapse by high-affinity serotonin transporters (5-HTT). The 5-HTT in the brain is among the most important sites of action for many currently used antidepressant treatments [96]. A functional 44 basepair insertion/deletion polymorphism in the promoter region of the *5-HTT* gene (*5-HTTLPR*) located on chromosome 17q11.2 has been associated with neurpsychiatric diagnoses and individual responses to antidepressant treatments. Although this polymorphism can be subdivided further [97], researchers commonly report it with two variations in humans: a long ("L") or a short ("S") variant allele. *In vitro* studies showed that basal transcriptional activity of the L allele is more than doubled when compared to the S variant allele [98]. Human individuals homozygous for the L/L variant show higher 5-HTT mRNA levels in postmortem brain tissue than subjects carrying the S allele (L/S + S/S) [99]. Moreover, reduced transcription associated with the S allele may affect serotonergic tone and 5-HT receptor-mediated neurotransmission [100]. An association study in 157 patients suffering from primary insomnia suggested that the S variant is over-represented in insomnia patients when compared to healthy controls (n = 827) [101]. Furthermore, this polymorphism may also mediate individual differences in the effects of chronic stress or stressful life events on impaired sleep quality and self-reported short sleep duration [102, 103]. Nevertheless, other research indicated poorer sleep in L/L homozygotes than in carriers of at least one S allele, suggesting that the effects of this gene may be heterogeneous in different populations [104].

*Genome-wide association (GWA) studies*

The Framingham Heart Study 100K Project revealed a linkage peak to usual sleep duration on chromosome 3 including the gene encoding prokineticin 2 (*PROK2*) [7]. This neuropeptide may be an important output molecule from the SCN, in particular in defining the onset and maintenance of the circadian night [105, 106]. Because the danger of false-positive inferences from small genome wide association studies is high, the methodological limitations of this work discussed above also apply to this potential association. It was not corroborated in a larger sample of the Framingham Cohort [8].

To identify novel genes associated with sleep duration, Allebrandt and colleagues performed GWA studies for self-reported average weekly sleep duration in seven discovery cohorts of a European consortium (n = 4'251) [34]. Meta-analysis revealed a genome-wide significant signal in the *ABCC9* (ATP-binding cassette, sub-family C member 9) gene locus (rs11046205) that encodes one subunit of the ATP-sensitive potassium ($K_{ATP}$) channel [34]. The finding from the discovery cohorts was replicated when an *in silico* (GWA data) sample as well as a subgroup population of a large de-novo (single genotyping) sample were additionally included in the meta-analysis. To confirm the role of *ABCC9* in modulating sleep duration, the homologue of this gene was knocked down in *Drosophila*, which shortened night time sleep duration. Approximately 5 % of the variance in sleep duration may be explained by this genetic variation in *ABCC9* [34]. In a candidate gene approach of another group tempting to replicate the proposed association, a significant association of the *ABCC9* gene with sleep duration was seen for a different polymorphism (rs11046209) and only in a rare homozygous genotype (n = 2) [107]. By contrast, the previously suggested polymorphism of *ABCC9* (rs11046205) was associated with depressive symptoms.

A very recent study combining 18 community-based cohorts including more than 47'000 individuals of European ancestry revealed a genome-wide significant association with polymorphisms in a gene located on chromosome 2 encoding the thyroid-specific transcription factor *PAX8* (paired box gene 8) [107a]. The finding was replicated in an African-American sample of

~4800 individual. While the finding is interesting, each copy of the minor allele only cause a estimated increase in usual sleep duration of approximately 3 minutes per and explains as little as 0.07 % of variance in sleep duration.

In conclusion, no GWA studies of habitual sleep duration in humans has yet been convincingly reproduced or explained a major portion of the variance in sleep length. Large sample sizes are needed for detecting genome-wide significant variants of genetically complex traits such as sleep duration. Thus, the phenotypic data in the available studies typically rely on questionnaire-derived, self-reported sleep duration or time in bed. These measures differ when assessed with different questionnaires, as well as when compared to objectively verified sleep duration, which may challenge the reliability and reproducibility of the currently available studies.

**Genetic basis of sleep-wake regulation: interaction between circadian and homeostatic systems**

Many of the traits and genes described above concern sleep-wake characteristics as assessed under baseline conditions. How these alterations in sleep characteristics relate to sleep-wake regulation and how they may lead to functional consequences remains largely unexplored. The available data, however, already indicate that the effects cross boundaries between sleep and wakefulness, and homeostatic and circadian aspects of sleep-wake regulation. For example, the polymorphisms in *PER3, ADORA2A,* and *COMT* affect the EEG in NREM sleep, REM sleep and wakefulness. To investigate whether these changes reflect changes in EEG generating mechanisms with or without a relation to sleep regulatory processes requires these processes to be challenged by, for example, sleep deprivation.

*Circadian clock genes*

Comparing the effects of sleep deprivation to *PER3*$^{4/4}$ individuals revealed that the increase in theta activity in the EEG during wakefulness was more rapid in carriers of the *PER3*$^{5/5}$ genotype [46]. In addition, in recovery sleep following total sleep deprivation, REM sleep was reduced in *PER3*$^{5/5}$ individuals. Finally, some data suggested that the increase in slow-wave energy after sleep restriction was slightly higher in adults carrying the *PER3*$^{5/5}$ genotype than in *PER3*$^{4/5}$ and *PER3*$^{4/4}$ allele carriers [108], and also the decline of cognitive performance during prolonged wakefulness and after sleep restriction differed as a function of the *PER3* genotype [109-111]. The differential susceptibility to the negative effects of sleep loss on waking performance was particularly pronounced in the second half of the circadian night and on tasks of executive functioning [109]. One interpretation of these data is that the VNTR polymorphism in *PER3* affects the dynamics of the homeostatic process, which then through its interaction with the circadian regulation of performance leads to differential sleep ability and vulnerability to the negative effects of sleep loss [109, 111]. Indeed, it has previously been shown that individuals not only differ with respect to baseline characteristics of sleep, but also in their response to sleep loss and that this vulnerability is a trait-like characteristic. The data suggest a contribution of *PER3* to individual tolerance to shift work and jet-lag, which are highly prevalent in society.

A 6-hour sleep deprivation in mice carrying the Pro384Arg mutation of *BHLHE41* resulted in a smaller rebound in both NREM sleep and REM sleep, and a smaller relative increase in EEG delta power when compared to control mice [90]. Furthermore, a functional variant (c.1086C>T) at another location in the same exon was studied in a DZ twin pair. The carrier of the variant was reported to have less recovery sleep following sleep deprivation and to produce fewer performance lapses during prolonged waking than the no-variant carrier. The variant reduced the ability of *BHLHE41* to suppress CLOCK/BMAL1 and NPAS2/BMAL1 transactivation *in vitro*, suggesting that genetic variants modifying the normal function of BHLHE41 may affect the homoestatic response to sleep deprivation [92].

*Adenosinergic neuromodulation*

Quantitative trait-locus analyses in inbred mouse strains revealed that a genomic region including *Ada* modifies the rate at which NREM sleep need accumulates during wakefulness [51]. Based on this observation, it was investigated whether human carriers of G/A and G/G genotypes of *ADA* respond differently to sleep deprivation [54, 112]. Bachmann and colleagues first systematically studied attention, learning, memory, executive functioning, and self-reported sleep duration in 245 healthy adults. It was found that heterozygous carriers of the variant allele (G/A genotype, n = 29) performed significantly worse on the d2 attention task than G/G homozygotes (n = 191). To test whether this difference reflected elevated sleep pressure, sleep and sleep EEG before and after sleep deprivation were recorded in two prospectively-matched groups of 11 G/A and 11 G/G genotypes. Corroborating two independent studies [53, 55], EEG delta activity and slow wave sleep were higher in G/A than in G/G genotype. In addition, sustained attention (d2 and psychomotor vigilance tasks) and vigor were reduced, while EEG alpha oscillations in waking, as well as sleepiness, fatigue, and α-amylase activity in saliva (a proposed biomarker of sleep drive) were increased throughout prolonged wakefulness [54]. These convergent behavioral, neurophysiological, subjective, and biochemical data demonstrated that genetically reduced ADA activity is associated with elevated sleep pressure. By contrast, the dynamics of the homeostatic response to sleep deprivation were not affected by *ADA* genotype [54, 112]. Thus, the data suggest an elevated level in overt NREM sleep propensity in the G/A genotype compared with G/G homozygotes, which may be due to elevated adenosinergic tone at the synapse because of genetically reduced ADA activity.

Convergent observations in candidate-gene and GWA studies strongly suggest that genetic variation of *ADORA2A* is a determinant of individual sensitivity to subjective and objective effects of caffeine on sleep [113, 114]. Interestingly, caffeine sensitive and insensitive individuals appeared to be differently affected by sleep loss [115]. These observations suggest that genetic variants of *ADORA2A* may alter the accumulation of homeostatically-regulated sleep propensity during prolonged wakefulness. Convergent findings in mice [116] and humans [117] are consistent with this notion. They

indicate that the sleep-deprivation-induced rebound of EEG delta activity in NREM sleep, the most reliable marker of sleep homeostasis, depends on the functional state of $A_{2A}$ receptors [118].

*Neurotransmitters*

Valomon *et al.* [119] recently investigated whether the Val158Met polymorphism of *COMT* (rs4680) affects actigraphy-derived rest-activity cycles and sleep estimates in 110 healthy adults. No genotype-dependent differences in actgraphy-derived circadian rest-activity patterns were found. Nevertheless, *COMT* genotype modulated the magnitude of sleep rebound on rest days when compared to work days. This difference is thought to reflect the compensation for a sleep debt accumulated during work days ('social jetlag'). The Val/Val and Met/Met homozygotes significantly prolonged habitual sleep on rest days, whereas the Val/Met heterozygotes did not [119]. Similarly, neurophysiological markers of sleep homeostasis did not differ between homozygous Val/Val and Met/Met allele carriers [63, 65]. By contrast, one study suggested that the Val158Met polymorphism of *COMT* may be related to inter-individual differences in sleep homeostasis and physiological sleep responses to partial sleep deprivation [120]. To further tackle the question whether COMT plays a role in sleep homeostasis, the effects of pharmacological interference with COMT enzymatic activity on the consequences of sleep deprivation in different *COMT* genotpyes may be studied.

*Transporters*

Genetically-modified animals with reduced dopamine clearance exhibit an increased homeostatic response to prolonged wakefulness when compared to wildtype animals. For example, mutant flies ($Dat^{Jo}$) with reduced *Dopamine acetyltransferase* activity show a greater sleep rebound after prolonged waking than wildtype controls [121]. Furthermore, *Drosophila* and mouse mutants lacking functional dopamine transporters (DAT) exhibit prolonged wakefulness and shortened sleep [122-124]. In mammals, the DAT is highly expressed in basal ganglia where it is responsible for re-uptake

of dopamine and constitutes a rate-limiting mechanism of dopaminergic neurotransmission [125]. An important role for the basal ganglia in sleep-wake regulation has been recently suggested [126, 127]. The response to sleep deprivation was studied in 57 adult volunteers genotyped for the 3'-UTR VNTR polymorphism of the gene (*DAT1*, *SLC6A3*) encoding DAT (rs28363170). Ten (10R) or 9 repeats (9R) of a 40-bp sequence of this gene on chromosome 5p15.3 are most common, whereas the 10R-allele homozygotes have 15-20 % reduced DAT availability in the striatum compared to hetero- and homozygous 9R-allele carriers [128, 129]. Consistent with the evidence from transgenic animals, it was found that the sleep deprivation-induced increase in SWS, EEG delta activity, and number, amplitude and slope of low-frequency (0.5-2.0 Hz) oscillations in NREM sleep was significantly larger in the 10R/10R genotype than in the 9R carrier genotype [59]. The data indicated an increased homeostatic response to sleep deprivation in 10R/10R allele carriers of *DAT1* when compared to 9R allele carriers.

*Signaling pathways*

Recent findings in rats suggested a causal relationship between BDNF and the regulation of EEG delta activity in NREM sleep [130, 131]. Inspired by these studies, the possible impact of the Val66Met polymorphism on the regulation of neurophysiological markers of sleep homeostasis was examined in humans [66]. Delta power in the first NREM sleep episode of a baseline, as well as of a recovery night after prolonged wakefulness, was specifically higher in Val/Val compared to Val/Met genotype subjects. By contrast, activity in high-alpha/low-sigma frequencies (~ 10-13.5 Hz range) was reduced. Thus, *BDNF* genotype modulated established EEG markers of NREM sleep intensity, whereas the rebound in delta activity after sleep deprivation and its dissipation throughout the nights were only subtly affected. These findings suggest that Val/Val genotypes exhibit overall higher NREM sleep pressure than Val/Met genotypes which may obscure subtle genotype-dependent differences in the dynamics of sleep homeostasis.

*Immune response*

The human leukocyte antigen (HLA) *DQB1*0602* allele is the best HLA marker for narcolepsy, a neurologic disorder characterized by excessive daytime sleepiness, fragmented sleep, and shortened REM sleep latency. Although over 90 % of patients with narcolepsy-cataplexy carry HLA-*DQB1*0602*, 12-38 % of allele-positive carriers are healthy sleepers [132, 133]. A study in 129 healthy subjects suggested that *DQB1*0602*-positive individuals showed decreased sleep homeostatic pressure with steeper declines and greater sleepiness and fatigue in baseline [134]. During partial sleep deprivation, slow-wave energy increased in positive and negative subjects, whereas *DQB1*0602*-positive positive individuals showed more fragmented sleep and altered REM and stage 2 sleep in baseline and during partial sleep loss. While these preliminary findings are interesting, independent replication is critically required for their validation.

**Human sleep pharmacogenetics**

Individual responses to treatments with pharmacological agents vary widely in healthy individuals and diseased patients. The differences may relate to weight, body composition, age, gender and ethnic descent. Furthermore, genetic factors modifying pharmacokinetic and/or pharmacodynamic properties of molecules and constitutive pathways are becoming increasingly recognized as key determinants of individual responses to pharmacological treatments. Apart from potentially important implications for the neurobiology of sleep-wake disorders and their pharmacological management, sleep pharmacogenetics also offers a powerful novel approach to identify molecular mechanisms contributing to sleep-wake regulation in humans. For example, pharmacogenetic studies of caffeine not only revealed insights into a distinct molecular contribution to individual caffeine sensitivity, but also indicate that adenosine $A_{2A}$ receptors and DAT are part of a biological pathway that regulates sleep.

*Adenosinergic neuromodulation*

Since people drink coffee, it is well-known that some people are sensitive to its stimulant effects whereas some others are not. With respect to sleep disturbances, already the first scientific study 100 years ago showed that "a few individuals show complete resistance to the effects of small doses of caffeine" [135]. Because subsequent work revealed no consistent pharmacokinetic differences between caffeine sensitive and insensitive subjects, endogenous diversity at its site of action was proposed to influence caffeine's effects on sleep [136]. Recent work in mice provided strong evidence that the stimulant promotes wakefulness primarily by blocking the $A_{2A}$ subtype of adenosine receptors [137]. In humans, the variant rs5751876 in the coding region of the *ADORA2A* gene contributes to individual sensitivity to caffeine effects on sleep [113]. In 4'329 responders to a brief internet questionnaire, caffeine consumption was associated with subjectively reduced sleep quality in caffeine sensitive respondents, but not in caffeine insensitive respondents, and the distribution of carriers of C/C and T/T alleles of *ADORA2A* differed between caffeine sensitive and insensitive individuals. Double-blind study of the effects of the stimulant on the sleep EEG confirmed the self-rated caffeine sensitivity, suggesting that genetic variation of *ADORA2A* is a determinant of individual sensitivity to the effects of caffeine on sleep [113].

Indeed, Byrne and colleagues [114] provided a recent confirmation of a role for *ADORA2A* in caffeine-related sleep disturbances. They conducted a GWA study in 2'402 twins and their families of the Australian Twin Registry. More than 2 million common polymorphisms were examined. Caffeine-associated sleep disturbance was based on the participants' report of whether or not they have ever experienced caffeine-induced insomnia, statistically corrected by a 'general insomnia factor score' derived from a questionnaire. Importantly, the previously suggested association between genetic variation of *ADORA2A* and disturbed sleep after caffeine was successfully replicated. This finding is remarkable in the genetics of complex traits because only a small minority of candidate genes has

typically been confirmed [138]. The original variant (rs5751876) was not typed in the GWA sample. Nevertheless, this variant forms a perfect linkage-disequilibrium with several other variants of *ADORA2A* that significantly affect caffeine-induced sleep disturbance [114].

Rétey *et al*. [113] combined self-reports and polysomnography after double-blind caffeine administration to document individual differences in the effects of caffeine on sleep. By contrast, the replication study was restricted to self-classification of caffeine sensitivity. The successful replication with this less accurate and less reliable (i.e., subjective) phenotype indicates that questionnaires are useful in large-scale epidemiological studies. Subsequent follow-up with objective measurements in animals and humans can provide novel insights into the molecular bases of healthy and disturbed sleep. Thus, sleep pharmacogenetics of caffeine may have important implications for the pathophysiology and the rational treatment of insomnia, as well as for recommendations for the critical use of caffeine, which is consumed on a daily basis by up to 90 % of adults in western societies.

*Dopaminergic neurotransmission*

Apart from being an adenosine receptor antagonist, the stimulant actions of caffeine also depend on the dopaminergic system. Data in *Dat* knock-out animals and human homozygous carriers of the 10R-allele of *DAT1* (*SLC6A3*) suggest that reduced DAT expression is associated with elevated sensitivity to the stimulant [59, 124]. Furthermore, Holst *et al.* [59] found that caffeine reduced distinct neurophysiological markers of sleep homeostasis, such as number, amplitude and slope of individual slow waves, in a *DAT1* genotype-dependent manner. This finding suggested that the interference of caffeine with neurophysiologic markers of sleep homeostasis not only relies on adenosinergic mechanisms, but involves also dopaminergic processes.

Caffeine alike, the potency of the wake-promoting compound modafinil shows pronounced inter-individual variation. The neurochemical mechanisms and cerebral regions through which

modafinil produces wakefulness are incompletely understood. However, modafinil reduces DAT-mediated re-uptake of dopamine in animals [139] and humans [140]. Consistent with a dopaminergic mode of action of modafinil, the compound was ineffective in promoting wakefulness in *Dat* knock-out mice [124] and attenuated elevated sleepiness after sleep deprivation reflected in EEG theta (5.5-7 Hz) power in sleep-deprived volunteers in a *DAT1* genotype-dependent manner [59].

Functional variants in the gene encoding COMT also alter dopaminergic neurotransmission in the brain. They may, thus, also contribute to individual differences in the wake-promoting effects of modafinil. Support for this hypothesis was obtained in both sleepy patients (see Clinical pearl) as well as in healthy volunteers subjected to sleep deprivation [141]. In healthy young men, placebo-controlled, double-blind, randomized administration of modafinil (2 x 100 mg) during prolonged wakefulness similarly reduced subjective sleepiness and EEG 5-8 Hz activity in Val/Val and Met/Met allele carriers of *COMT* [63]. By contrast, modafinil differently affected the NREM sleep EEG in recovery sleep. Furthermore, it maintained sustained vigilant attention and executive functioning at baseline level throughout prolonged waking in Val/Val allele carriers, whereas the compound was virtually ineffective in the Met/Met genotype [65]. These data highlight a role for dopamine in impaired waking functions after sleep loss. The functional significance of the modafinil-induced, genotype-dependent effects on the NREM sleep EEG during recovery from sleep loss remains to be determined.

**Concluding remarks**

Sleep is a complex behavior and any functional genetic variation associated with changes in one of the many neurotransmitter/neuromodulator system can be expected to affect sleep and the sleep EEG. Polymorphic variations in a number of genes have now been shown to affect several characteristics of sleep and some of these genes may indeed be involved in sleep regulatory processes. However, many associations need to be replicated and failure of replication is common. Nevertheless, once robust associations have been established, elucidating the signaling pathways that are affected will

aid to our understanding of individual differences in sleep-wake behavior.

**Clinical pearl**

Distinct alleles and genotypes in the genes of monoamine oxidase type A (*MAO-A*) [142] - but see [143] – and *COMT* [143] are thought to be associated with the clinical manifestation of narcolepsy. The Val158Met polymorphism of *COMT* exerts a sexual dimorphism and a strong effect of genotype on disease severity [143]. More specifically, women narcoleptics with high COMT activity fall asleep twice as fast during the multiple sleep latency test than those with low COMT activity. An opposite relationship, although less pronounced, is observed in men. Also the response to treatment with modafinil to control excessive daytime sleepiness differs between *COMT* genotypes. Patients (female and male) with the Val/Val genotype need a an almost 100 mg higher daily dose than patients with the Met/Met genotype [144]. Intriguingly, in male healthy volunteers, the impact of the Val158Met polymorphism of *COMT* on modafinil's efficacy to improve excessive sleepiness after sleep deprivation is opposite to that in narcolepsy patients [65].

**Summary**

Sleep is a very rich phenotype and many aspects of sleep differ considerably in the population of healthy individuals (even when only a very narrow age range is considered). Inter-individual variation in sleep timing (diurnal preference), sleep duration, sleep structure and the EEG in NREM sleep, REM sleep and wakefulness, have all been shown to have a genetic basis. The response to challenges of sleep regulatory processes such as sleep deprivation and circadian misalignment has also been shown to vary between individuals. Some of the polymorphic variations in genes contributing to variation in sleep characteristics have now been identified. They include variations in genes associated with the circadian system (e.g., *CLOCK, PER1, PER2, PER3, BHLHE41*), the adenosine system (*ADA, ADORA2A*) and catecholaminergic system (e.g., *COMT, SLC6A3, SLC6A4*),

as well as other signaling pathways (e.g., *ABCC9*, *BDNF*, *PRNP*) For some of these genes, so far only associations with one aspect of sleep have been reported, e.g., *PER2* and sleep timing. Variations in other genes have been shown to affect multiple aspects of sleep and wakefulness, as well as the response to sleep loss or pharmacological interventions. For example, *PER3* and *ADA* affect the EEG and performance during prolonged waking, whereas *ADORA2A*, *COMT* and *SLC6A3* modulate EEG and response to the stimulants caffeine and modafinil. All currently known polymorphic variations explain only a small part of the variation in healthy human sleep phenotypes, and many more genetic contributions remain to be discovered.

**Selected Readings**

Andretic, R., Franken, P. & Tafti, M. Genetics of Sleep. *Annu Rev Genet* **42**, 361-388 (2008).

Lazar, A.S*., et al.* Sleep, Diurnal Preference, Health, and Psychological Well-being: A Prospective Single-Allelic-Variation Study. *Chronobiol Int* **29**, 131-146 (2012).

Hida, A*., et al.* Screening of clock gene polymorphisms demonstrates association of a PER3 polymorphism with morningness-eveningness preference and circadian rhythm sleep disorder. *Sci Rep* **4**, 6309 (2014).

Byrne, E.M*., et al.* A genome-wide association study of sleep habits and insomnia. *Am J Med Genet B Neuropsychiatr Genet* **162B**, 439-451 (2013).

Landolt, H.-P. Genetic determinants of sleep EEG profiles in healthy humans. *Prog Brain Res* **193**, 51-61 (2011).

De Gennaro, L*., et al.* The EEG fingerprint of sleep is genetically determined: a twin study. *Ann Neurol* **64**, 455-460 (2008).

Dijk, D.J. & Archer, S.N. PERIOD3, circadian phenotypes, and sleep homeostasis. *Sleep Med Rev* **14**, 151-160 (2010).

Mang, G.M. & Franken, P. Genetic Dissection of Sleep Homeostasis. *Curr Top Behav Neurosci*, Epub ahead of print (2013).

Byrne, E.M.*, et al.* A Genome-Wide Association Study of Caffeine-Related Sleep Disturbance: Confirmation of a Role for a Common Variant in the Adenosine Receptor. *Sleep* **35**, 967-975 (2012).

McCarthy, M.I.*, et al.* Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nature Rev Genet* **9**, 356-369 (2008).

Dauvilliers, Y., Tafti, M. & Landolt, H.-P. Catechol-O-methyltransferase, dopamine, and sleep-wake regulation. *Sleep Med Rev*, in press (2015).

**Acknowledgements**

The authors' research has been supported by Swiss National Science Foundation, Zürich Center for interdisciplinary Sleep Research, Clinical Research Priority Program "Sleep & Health" of the University of Zürich, Zürich Center for Integrative Human Physiology, Neuroscience Center Zürich, and Novartis Foundation for Medical-Biological Research (to HPL), and Biotechnology and Biological Sciences Research Council, Wellcome Trust, the Airforce Office of Scientific Research, the Higher Education Funding Council for England and a Wolfson-Royal Society Award (to DJD).

**References**

1. Andretic, R., Franken, P. & Tafti, M. Genetics of Sleep. *Annu Rev Genet* **42**, 361-388 (2008).
2. Tucker, A.M., Dinges, D.F. & Van Dongen, H.P.A. Trait interindividual differences in the sleep physiology of healthy young adults. *J Sleep Res* **16**, 170-180 (2007).
3. Adan, A.*, et al.* Circadian Typology: A Comprehensive Review. *Chronobiol Int* **29**, 1153-1175 (2012).
4. Kripke, D.F., Simons, R.N., Garfinkel, L. & Hammond, E.C. Short and long sleep and sleeping pills. Is increased mortality associated? *Arch Gen Psychiatry* **36**, 103-116 (1979).
5. Mishima, K., Tozawa, T., Satoh, K., Saitoh, H. & Mishima, Y. The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. *Am J Med Genet B Neuropsychiatr Genet* **133B**, 101-104 (2005).
6. Koskenvuo, M., Hublin, C., Partinen, M., Heikkila, K. & Kaprio, J. Heritability of diurnal type: a nationwide study of 8753 adult twin pairs. *J Sleep Res* **16**, 156-162 (2007).
7. Gottlieb, D.J., O'Connor, G.T. & Wilk, J.B. Genome-wide association of sleep and circadian phenotypes. *BMC Med Genet* **8**, S9 (2007).
8. Chen, T., Wilk, J.B., O'Connor, G.T. & Gottlieb, D.J. Genetics of sleep phenotypes in the NHLBI's Framingham Heart Study offspring cohort. *Sleep* **32**, A401 (2009).
9. Duffy, J.F., Rimmer, D.W. & Czeisler, C.A. Association of intrinsic circadian period with morningness-eveningness, usual wake time, and circadian phase. *Behav Neurosci* **115**, 895-899 (2001).
10. Wright, K.P., Gronfier, C., Duffy, J.E. & Czeisler, C.A. Intrinsic period and light intensity determine the phase relationship between melatonin and sleep in humans. *J Biol Rhythms* **20**, 168-177 (2005).
11. Lazar, A.S.*, et al.* Circadian period and the timing of melatonin onset in men and women: predictors of sleep during the weekend and in the laboratory. *J Sleep Res* **22**, 155-159 (2013).

12. Hasan, S*., et al.* Assessment of circadian rhythms in humans: comparison of real-time fibroblast reporter imaging with plasma melatonin. *Faseb J* **26**, 2414-2423 (2012).

13. Beelman, C.A. & Parker, R. Degradation of messenger-RNA in Eukaryotes. *Cell* **81**, 179-183 (1995).

14. Katzenberg, D*., et al.* A CLOCK polymorphism associated with human diurnal preference. *Sleep* **21**, 569-576 (1998).

15. Robilliard, D.L*., et al.* The 3111 Clock gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. *J Sleep Res* **11**, 305-312 (2002).

16. Pedrazzoli, M*., et al.* Clock polymorphisms and circadian rhythms phenotypes in a sample of the Brazilian population. *Chronobiol Int* **24**, 1-8 (2007).

17. Bae, K*., et al.* Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. *Neuron* **30**, 525-536 (2001).

18. Katzenberg, D., Young, T., Lin, L., Finn, L. & Mignot, E. A human period gene (HPER1) polymorphism is not associated with diurnal preference in normal adults. *Psychiatr Genet* **9**, 107-109 (1999).

19. Carpen, J.D., von Schantz, M., Smits, M., Skene, D.J. & Archer, S.N. A silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. *J Hum Genet* **51**, 1122-1125 (2006).

20. Lim, A.S.P*., et al.* A common polymorphism near PER1 and the timing of human behavioral rhythms. *Ann Neurol* **72**, 324-334 (2012).

21. Toh, K.L*., et al.* An hPer2 phosphorylation site mutation in familiar advanced sleep phase syndrome. *Science* **291**, 1040-1043 (2001).

22. Xu, Y*., et al.* Functional consequences of a CKI delta mutation causing familial advanced sleep phase syndrome. *Nature* **434**, 640-644 (2005).

23. Xu, Y.*, et al.* Modeling of a human circadian mutation yields insights into clock regulation by PER2. *Cell* **128**, 59-70 (2007).

24. Carpen, J.D., Archer, S.N., Skene, D.J., Smits, M. & von Schantz, M. A single-nucleotide polymorphism in the 5 '-untranslated region of the hPER2 gene is associated with diurnal preference. *J Sleep Res* **14**, 293-297 (2005).

25. Ramanathan, C.*, et al.* Cell Type-Specific Functions of Period Genes Revealed by Novel Adipocyte and Hepatocyte Circadian Clock Models. *Plos Genet* **10** (2014).

26. Archer, S.N.*, et al.* A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* **26**, 413-415 (2003).

27. Pereira, D.S.*, et al.* Association of the length polymorphism delayed sleep-phase syndrome: Does in the human Per3 gene with the latitude have an influence upon it? *Sleep* **28**, 29-32 (2005).

28. Lazar, A.S.*, et al.* Sleep, Diurnal Preference, Health, and Psychological Well-being: A Prospective Single-Allelic-Variation Study. *Chronobiol Int* **29**, 131-146 (2012).

29. Hida, A.*, et al.* Screening of clock gene polymorphisms demonstrates association of a PER3 polymorphism with morningness-eveningness preference and circadian rhythm sleep disorder. *Sci Rep* **4**, 6309 (2014).

30. Parsons, M.J.*, et al.* Polymorphisms in the circadian expressed genes PER3 and ARNTL2 are associated with diurnal preference and GNbeta3 with sleep measures. *J Sleep Res* **23**, 595-604 (2014).

31. Hohjoh, H.*, et al.* Significant association of the arylalkylamine N-acetyltransferase (AA-NAT) gene with delayed sleep phase syndrome. *Neurogenet* **4**, 151-153 (2003).

32. Pereira, D.S.*, et al.* The G619A Aa-nat gene polymorphism does not contribute to sleep time variation in the brazilian population. *Behav Genet* **37**, 637-638 (2007).

33. Wang, G.Y., Lee, C.G.L. & Lee, E.J.D. Genetic variability of arylalkylamine-N-acetyl-transferase (AA-NAT) gene and human sleep/wake pattern. *Chronobiol Int* **21**, 229-237 (2004).

34. Allebrandt, K.V.*, et al.* A K-ATP channel gene effect on sleep duration: from genome-wide association studies to function in Drosophila. *Mol Psychiatry* **18**, 122-132 (2013).

35. Byrne, E.M.*, et al.* A genome-wide association study of sleep habits and insomnia. *Am J Med Genet B Neuropsychiatr Genet* **162B**, 439-451 (2013).

36. Reinscheid, R.K.*, et al.* Pharmacological characterization of human and murine neuropeptide S receptor variants. *J Pharmacol Exp Ther* **315**, 1338-1345 (2005).

37. Borbély, A.A., Baumann, F., Brandeis, D., Strauch, I. & Lehmann, D. Sleep deprivation: effect on sleep stages and EEG power density in man. *Electroencephalogr clin Neurophysiol* **51**, 483-493 (1981).

38. Geering, B.A., Achermann, P., Eggimann, F. & Borbély, A.A. Period-amplitude analysis and power spectral analysis: a comparison based on all-night sleep EEG recordings. *J Sleep Res* **2**, 121-129 (1993).

39. Tan, X., Campbell, I.G. & Feinberg, I. Internight reliability and benchmark values for computer analyses of non-rapid eye movement (NREM) and REM EEG in normal young adult and elderly subjects. *Clin Neurophysiol* **112**, 1540-1552 (2001).

40. Buckelmüller, J., Landolt, H.P., Stassen, H.H. & Achermann, P. Trait-like individual differences in the human sleep electroencephalogram. *Neuroscience* **138**, 351-356 (2006).

41. Ambrosius, U.*, et al.* Heritability of sleep electroencephalogram. *Biol Psychiatry* **64**, 344-348 (2008).

42. Landolt, H.-P. Genetic determinants of sleep EEG profiles in healthy humans. *Prog Brain Res* **193**, 51-61 (2011).

43. De Gennaro, L.*, et al.* The EEG fingerprint of sleep is genetically determined: a twin study. *Ann Neurol* **64**, 455-460 (2008).

44. Franken, P. A role for clock genes in sleep homeostasis. *Curr Opin Neurobiol* **23**, 864-872 (2013).

45. Dijk, D.J. & Archer, S.N. PERIOD3, circadian phenotypes, and sleep homeostasis. *Sleep Med Rev* **14**, 151-160 (2010).

46. Viola, A.U.*, et al.* PER3 polymorphism predicts sleep structure and waking performance. *Curr Biol* **17**, 613-618 (2007).

47. Viola, A.U.*, et al.* Interindividual differences in circadian rhythmicity and sleep homeostasis in older people: effect of a PER3 polymorphism. *Neurobiol Aging* **33** (2012).

48. Landolt, H.P. Sleep homeostasis: A role for adenosine in humans? *Biochem Pharmacol* **75**, 2070-2079 (2008).

49. Fredholm, B.B., Chen, J.F., Cunha, R.A., Svenningsson, P. & Vaugeois, J.M. Adenosine and brain function. *Int Rev Neurobiol* **63**, 191-270 (2005).

50. Palchykova, S.*, et al.* Manipulation of Adenosine Kinase Affects Sleep Regulation in Mice. *J Neurosci* **30**, 13157-13165 (2010).

51. Franken, P., Chollet, D. & Tafti, M. The homeostatic regulation of sleep need is under genetic control. *J Neurosci* **21**, 2610-2621. (2001).

52. Riksen, N.P.*, et al.* The 22G > A polymorphism in the adenosine deaminase gene impairs catalytic function but does not affect reactive hyperaemia in humans in vivo. *Pharmacogenet Genom* **18**, 843-846 (2008).

53. Rétey, J.V.*, et al.* A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. *Proc Natl Acad Sci USA* **102**, 15676-15681 (2005).

54. Bachmann, V.*, et al.* Functional ADA Polymorphism Increases Sleep Depth and Reduces Vigilant Attention in Humans. *Cereb Cortex* **22**, 962-970 (2012).

55. Mazzotti, D.R.*, et al.* Adenosine Deaminase Polymorphism Affects Sleep EEG Spectral Power in a Large Epidemiological Sample. *PLoS ONE* **7** (2012).

56. Sebastiao, A.M. & Ribeiro, J.A. Adenosine receptors and the central nervous system. In: *Handbook of Experimental Pharmacology* (ed. C.N. Wilson & S.J. Mustafa) 471-534 (Springer Verlag, Berlin Heidelberg, 2009).

57. Alsene, K., Deckert, J., Sand, P. & de Wit, H. Association between A(2a) receptor gene polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacol* **28**, 1694-1702 (2003).

58. Volkow, N.D.*, et al.* Evidence That Sleep Deprivation Downregulates Dopamine D2R in Ventral Striatum in the Human Brain. *J Neurosci* **32**, 6711-6717 (2012).

59. Holst, S.C.*, et al.* Dopaminergic Role in Regulating Neurophysiological Markers of Sleep Homeostasis in Humans. *J Neurosci* **34**, 566-573 (2014).

60. Lachman, H.M.*, et al.* Human catechol-O-methyltransferase pharmacogenetics: Description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenet* **6**, 243-250 (1996).

61. Chen, J.S.*, et al.* Functional analysis of genetic variation in catechol-o-methyltransferase (COMT): Effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* **75**, 807-821 (2004).

62. Meyer-Lindenberg, A.*, et al.* Impact of complex genetic variation in COMT on human brain function. *Mol Psychiatry* **11**, 867-877 (2006).

63. Bodenmann, S. & Landolt, H.P. Effects of Modafinil on the Sleep EEG Depend on Val158Met Genotype of COMT. *Sleep* **33**, 1027-1035 (2010).

64. Bodenmann, S.*, et al.* The Functional Val158Met Polymorphism of COMT Predicts Inter-individual Differences in Brain alpha Oscillations in Young Men. *J Neurosci* **29**, 10855-10862 (2009).

65. Bodenmann, S.*, et al.* Pharmacogenetics of Modafinil After Sleep Loss: Catechol-O-Methyl-transferase Genotype Modulates Waking Functions But Not Recovery Sleep. *Clin Pharmacol Ther* **85**, 296-304 (2009).

66. Bachmann, V.*, et al.* The BDNF Val66Met polymorphism modulates sleep intensity: EEG frequency- and state-specificity. *Sleep* **35**, 335-344 (2012).

67. Guindalini, C.*, et al.* Brain-Derived Neurotrophic Factor Gene Polymorphism Predicts Inter-individual Variation in the Sleep Electroencephalogram. *J Neurosci Res* **92**, 1018-1023 (2014).

68. Egan, M.F.*, et al.* The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257-269 (2003).

69. Lugaresi, E.*, et al.* Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *New Engl J Med* **315**, 997-1003 (1986).

70. Montagna, P., Gambetti, P., Cortelli, P. & Lugaresi, E. Familial and sporadic fatal insomnia. *Lancet Neurol* **2**, 167-176 (2003).

71. Ferrillo, F.*, et al.* Absence of sleep EEG markers in fatal familial insomnia healthy carriers: a spectral analysis study. *Clin Neurophysiol* **112**, 1888-1892 (2001).

72. Plazzi, G.*, et al.* Does the prion protein gene 129 codon polymorphism influence sleep? Evidence from a fatal familial insomnia kindred. *Clin Neurophysiol* **113**, 1948-1953 (2002).

73. Merica, H. & Gaillard, J.M. Statistical Description and Evaluation of the Interrelationships of Standard Sleep Variables for Normal Subjects. *Sleep* **8**, 261-273 (1985).

74. Zung, W.W. & Wilson, W.P. Sleep and dream patterns in twins. Markov analysis of a genetic trait. *Recent Adv Biol Psychiatry* **9**, 119-130 (1966).

75. Webb, W.B. & Campbell, S.S. Relationships in Sleep Characteristics of Identical and Fraternal-Twins. *Arch Gen Psychiatry* **40**, 1093-1095 (1983).

76. Chouvet, G., Blois, R., Debilly, G. & Jouvet, M. La structure d'occurrence des mouvements oculaires rapides du sommeil paradoxal est similaire chez les jumeaux homozygotes. *C R Acad Sc Paris III* **296**, 1063-1068 (1983).

77. Linkowski, P. EEG sleep patterns in twins. *J Sleep Res* **8**, Suppl 1, 11-13 (1999).

78. Chellappa, S.L.*, et al.* Light modulation of human sleep depends on a polymorphism in the clock gene Period3. *Behav Brain Res* **271**, 23-29 (2014).

79. Aeschbach, D., Cajochen, C., Landolt, H.P. & Borbély, A.A. Homeostatic sleep regulation in habitual short sleepers and long sleepers. *Am J Physiol* **270**, R41-R53 (1996).

80. Aeschbach, D.*, et al.* Evidence from the waking electroencephalogram that short sleepers live under higher homeostatic sleep pressure than long sleepers. *Neuroscience* **102**, 493-502 (2001).

81. Aeschbach, D.*, et al.* A longer biological night in long sleepers than in short sleepers. *J Clin Endocrinol Metab* **88**, 26-30 (2003).

82. Groeger, J.A., Zijlstra, F.R.H. & Dijk, D.J. Sleep quantity, sleep difficulties and their perceived consequences in a representative sample of some 2000 British adults. *J Sleep Res* **13**, 359-371 (2004).

83. Landolt, H.P. Genotype-Dependent Differences in Sleep, Vigilance, and Response to Stimulants. *Curr Pharm Design* **14**, 3396-3407 (2008).

84. Gedda, L. & Brenci, G. Sleep and dream characteristics in twins. *Acta Genet Med Gemellol*, 237-239 (1979).

85. Partinen, M., Kaprio, J., Koskenvuo, M., Putkonen, P. & Langinvainio, H. Genetic and environmental determination of human sleep. *Sleep* **6**, 179-185 (1983).

86. Heath, A.C., Kendler, K.S., Eaves, L.J. & Martin, N.G. Evidence for genetic influences on sleep disturbance and sleep pattern in twins. *Sleep* **13**, 318-335 (1990).

87. Allebrandt, K.V.*, et al.* CLOCK Gene Variants Associate with Sleep Duration in Two Independent Populations. *Biol Psychiatry* **67**, 1040-1047 (2010).

88. Evans, D.S.*, et al.* Common Genetic Variants in ARNTL and NPAS2 and at Chromosome 12p13 are Associated with Objectively Measured Sleep Traits in the Elderly. *Sleep* **36**, 431-446 (2013).

89. Lane, J.M.*, et al.* Common Variants in CLOCK Are Not Associated with Measures of Sleep Duration in People of European Ancestry from the Sleep Heart Health Study. *Biol Psychiatry* **74**, e33-e35 (2013).

90. He, Y.*, et al.* The Transcriptional Repressor DEC2 Regulates Sleep Length in Mammals. *Science* **325**, 866-870 (2009).

91. Mang, G.M. & Franken, P. Genetic Dissection of Sleep Homeostasis. *Curr Top Behav Neurosci*, Epub ahead of print (2013).

92. Pellegrino, R.*, et al.* A Novel BHLHE41 Variant is Associated with Short Sleep and Resistance to Sleep Deprivation in Humans. *Sleep* **37**, 1327-1336 (2014).

93. Utge, S.*, et al.* Shared Genetic Background for Regulation of Mood and Sleep: Association of GRIA3 with Sleep Duration in Healthy Finnish Women. *Sleep* **34**, 1309-U1341 (2011).

94. Jouvet, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergeb Physiol* **64**, 166-307 (1972).

95. Jones, B.E. Basic mechanisms of sleep-wake states. In: *Principles and Practise of Sleep Medicine* (ed. M.H. Kryger, T. Roth & W.C. Dement) 136-153 (Elsevier Saunders, Philadelphia, PA, 2005).

96. Hu, X.Z.*, et al.* Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. *Arch Gen Psychiatry* **64**, 783-792 (2007).

97. Nakamura, M., Ueno, S., Sano, A. & Tanabe, H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol Psychiatry* **5**, 32-38 (2000).

98. Lesch, K.P.*, et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527-1531 (1996).

99. Little, K.Y.*, et al.* Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *Am J Psychiatry* **155**, 207-213 (1998).

100. David, S.P.*, et al.* A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. *J Neurosci* **25**, 2586-2590 (2005).

101. Deuschle, M.*, et al.* Association between a Serotonin Transporter Length Polymorphism and Primary Insomnia. *Sleep* **33**, 343-347 (2010).

102. Brummett, B.H.*, et al.* Sleep quality varies as a function of 5-HTTLPR genotype and stress. *Psychosom Med* **69**, 621-624 (2007).

103. Carskadon, M.A., Sharkey, K.M., Knopik, V.S. & McGeary, J.E. Short Sleep as an Environmental Exposure: A Preliminary Study Associating 5-HTTLPR Genotype to Self-Reported Sleep Duration and Depressed Mood in First-Year University Students. *Sleep* **35**, 791-796 (2012).

104. Barclay, N.L*., et al.* Sleep Quality and Diurnal Preference in a Sample of Young Adults: Associations With 5HTTLPR, PER3, and CLOCK 3111. *Am J Med Genet B Neuropsychiatr Genet* **156B**, 681-690 (2011).

105. Cheng, M.Y*., et al.* Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* **417**, 405-410 (2002).

106. Prosser, H.M*., et al.* Prokineticin receptor 2 (Prokr2) is essential for the regulation of circadian behavior by the suprachiasmatic nuclei. *Proc Natl Acad Sci USA* **104**, 648-653 (2007).

107. Parsons, M.J*., et al.* Replication of Genome-Wide association studies (GWAS) loci for sleep in the British G1219 cohort. *Am J Med Genet B Neuropsychiatr Genet* **162B**, 431-438 (2013).

107a. Gottlieb, D.J., et al. Novel loci associated with usual sleep duration: the CHARGE Consortium Genome-Wide Association Study. *Mol Psychiatry*, in press (2015).

108. Goel, N., Banks, S., Mignot, E. & Dinges, D.F. PER3 Polymorphism Predicts Cumulative Sleep Homeostatic but Not Neurobehavioral Changes to Chronic Partial Sleep Deprivation. *PLoS ONE* **4**, e5874 (2009).

109. Groeger, J.A*., et al.* Early morning executive fuctioning during sleep deprivation is compromised by a *PERIOD3* polymorphism. *Sleep* **31**, 1159-1167 (2008).

110. Rupp, T.L., Wesensten, N.J., Newman, R. & Balkin, T.J. PER3 and ADORA2A polymorphisms impact neurobehavioral performance during sleep restriction. *J Sleep Res* **22**, 160-165 (2013).

111. Maire, M*., et al.* Sleep ability mediates individual differences in the vulnerability to sleep loss: Evidence from a PER3 polymorphism. *Cortex* **52**, 47-59 (2014).

112. Reichert, C.F*., et al.* Insights into Behavioral Vulnerability to Differential Sleep Pressure and Circadian Phase from a Functional ADA Polymorphism. *J Biol Rhythms* **29**, 119-130 (2014).

113. Rétey, J.V.*, et al.* A Genetic Variation in the Adenosine $A_{2A}$ Receptor Gene (*ADORA2A*) Contributes to Individual Sensitivity to Caffeine Effects on Sleep. *Clin Pharmacol Ther* **81**, 692-698 (2007).

114. Byrne, E.M.*, et al.* A Genome-Wide Association Study of Caffeine-Related Sleep Disturbance: Confirmation of a Role for a Common Variant in the Adenosine Receptor. *Sleep* **35**, 967-975 (2012).

115. Rétey, J.V.*, et al.* Adenosinergic mechanisms contribute to individual differences in sleep-deprivation induced changes in neurobehavioral function and brain rhythmic activity. *J Neurosci* **26**, 10472-10479 (2006).

116. Hayaishi, O., Urade, Y., Eguchi, N. & Huang, Z.-L. Genes for prostaglandin D synthase and receptor as well as adenosine $A_{2A}$ receptor are involved in the homeostatic regulation of NREM sleep. *Arch Ital Biol* **142**, 533-539 (2004).

117. Bodenmann, S.*, et al.* Polymorphisms of *ADORA2A* modulate psychomotor vigilance and the effects of caffeine on neurobehavioral performance and sleep EEG after sleep deprivation. *Br J Pharmacol* **165**, 1904-1913 (2012).

118. Landolt, H.P. "No Thanks, Coffee Keeps Me Awake": Individual Caffeine Sensitivity Depends on ADORA2A Genotype. *Sleep* **35**, 899-900 (2012).

119. Valomon, A.*, et al.* Genetic polymorphisms of *DAT* and *COMT* differently influence actigraphy-derived activity-rest cycles and sleep measures in humans. *Chronobiol Int* **31**, 705-714 (2014).

120. Goel, N., Banks, S., Lin, L., Mignot, E. & Dinges, D.F. Catechol-O-Methyltransferase Val158Met Polymorphism Associates with Individual Differences in Sleep Physiologic Responses to Chronic Sleep Loss. *PLoS ONE* **6** (2011).

121. Greenspan, R.J., Tononi, G., Cirelli, C. & Shaw, P.J. Sleep and the fruit fly. *Trends Neurosci* **24**, 142-145 (2001).

122. Kume, K., Kume, S., Park, S.K., Hirsh, J. & Jackson, F.R. Dopamine is a regulator of arousal in the fruit fly. *J Neurosci* **25**, 7377-7384 (2005).

123. Giros, B., Jaber, M., Jones, S.R., Wightman, R.M. & Caron, M.G. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **379**, 606-612 (1996).

124. Wisor, J.P*., et al.* Dopaminergic role in stimulant-induced wakefulness. *J Neurosci* **21**, 1787-1794 (2001).

125. Benoit-Marand, M., Jaber, M. & Gonon, F. Release and elimination of dopamine in vivo in mice lacking the dopamine transporter: functional consequences. *Eur J Neurosci* **12**, 2985-2992 (2000).

126. Lazarus, M., Chen, J.-F., Urade, Y. & Huang, Z.-L. Role of the basal ganglia in the control of sleep and wakefulness. *Curr Opin Neurobiol* **23**, 1-6 (2013).

127. Qiu, M.-H., Yao, Q.-L., Vetrivelan, R., Chen, M.C. & Lu, J. Nigrostriatal dopamine acting on globus pallidus regulates sleep. *Cereb Cortex*, in press (2014).

128. Costa, A., Riedel, M., Muller, U., Moller, H.J. & Ettinger, U. Relationship Between SLC6A3 Genotype and Striatal Dopamine Transporter Availability: A Meta-Analysis of Human Single Photon Emission Computed Tomography Studies. *Synapse* **65**, 998-1005 (2011).

129. Spencer, T.J*., et al.* Functional Genomics of Attention-Deficit/Hyperactivity Disorder (ADHD) Risk Alleles on Dopamine Transporter Binding in ADHD and Healthy Control Subjects. *Biol Psychiatry* **74**, 84-89 (2013).

130. Huber, R., Tononi, G. & Cirelli, C. Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep* **30**, 129-139 (2007).

131. Faraguna, U., Vyazovskiy, V.V., Nelson, A.B., Tononi, G. & Cirelli, C. A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. *J Neurosci* **28**, 4088-4095 (2008).

132. Mignot, E., Young, T., Lin, L. & Finn, L. Nocturnal sleep and daytime sleepiness in normal subjects with HLA-DQB1*0602. *Sleep* **22**, 347-352 (1999).

133. Dauvilliers, Y. & Tafti, M. Molecular genetics and treatment of narcolepsy. *Ann Med* **38**, 252-262 (2006).

134. Goel, N., Banks, S., Mignot, E. & Dinges, D.F. DQB1*0602 predicts interindividual differences in physiologic sleep, sleepiness, and fatigue. *Neurology* **75**, 1509-1519 (2010).

135. Hollingworth, H.L. The influence of caffein alkaloid on the quality and amount of sleep. *Am J Psychol* **23**, 89-100 (1912).

136. Goldstein, A., Warren, R. & Kaizer, S. Psychotropic effects of caffeine in man. I. Individual differences in sensitivity to caffeine-induced wakefulness. *J Pharmacol Exp Ther* **149**, 156-159 (1965).

137. Huang, Z.L.*, et al.* Adenosine $A_{2A}$, but not $A_1$, receptors mediate the arousal effect of caffeine. *Nature Neurosci* **8**, 858-859 (2005).

138. McCarthy, M.I.*, et al.* Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nature Rev Genet* **9**, 356-369 (2008).

139. Mignot, E., Nishino, S., Guilleminault, C. & Dement, W.C. Modafinil binds to the dopamine uptake carrier site with low affinity. *Sleep* **17**, 436-437 (1994).

140. Volkow, N.D.*, et al.* Effects of Modafinil on Dopamine and Dopamine Transporters in the Male Human Brain Clinical Implications. *JAMA* **301**, 1148-1154 (2009).

141. Dauvilliers, Y., Tafti, M. & Landolt, H.-P. Catechol-O-methyltransferase, dopamine, and sleep-wake regulation. *Sleep Med Rev*, in press (2015).

142. Koch, H., Craig, I., Dahlitz, M., Denney, R. & Parkes, D. Analysis of the monoamine oxidase genes and the Norrie disease gene locus in narcolepsy. *Lancet* **353**, 645-646 (1999).

143. Dauvilliers, Y., Neidhart, E., Lecendreux, M., Billiard, M. & Tafti, M. MAO-A and COMT polymorphisms and gene effects in narcolepsy. *Mol Psychiatry* **6**, 367-372 (2001).

144. Dauvilliers, Y., Neidhart, E., Billiard, M. & Tafti, M. Sexual dimorphism of the catechol-O-methyltransferase gene in narcolepsy is associated with response to modafinil. *Pharmaco-genomics J* **2**, 65-68 (2002).

**Table 1.** Genes investigated to contribute to genotype-dependent differences in diurnal preference, sleep timing, sleep EEG, sleep structure and sleep duration

| Family | Gene | NCBI SNP-ID | Base change / Amino acid change | Diurnal preference | Sleep timing | Sleep EEG | Sleep structure | Sleep duration | Sleep homeostasis | Reference(s) |
|---|---|---|---|---|---|---|---|---|---|---|
| Clock gene pathway | *CLOCK* | rs1801260 | c.3111T>C | √ | √ | √ | | | | 5, 14-16 |
| | *CLOCK* | rs2070062 | c.257T>G | √ | | | | | | 16 |
| | *CLOCK* | rs12649507 | | | | | | √ | | 87-89 |
| | *PER1* | | c.2548G>A | √ | | | | | | 18, 19 |
| | *PER1* | rs2735611 | c.2434T>C | √ | | | | | | 19 |
| | *PER1* | rs7221412 | g.8137696A>G | √ | | | | | | 20 |
| | *PER2* | | c.1984A>G / p.Ser662Gly | | √ | | | | | 21 |
| | *PER2* | rs2304672 | c.111G>C | √ | √ | | | | | 24 |
| | *PER3* | rs57875989 | VNTR / del(1011-1028 aa) | √ | √ | √ | √ | √ | √ | 46, 47, 108 |
| | *PER3* | rs228697 | c.2590C>G / p.Pro864Ala | √ | | | | | | 29 |
| | *PER3* | rs10462020 | c.1940T>G / p.Val647Gly | √ | | | | | | 30 |
| | *AANAT* | | c.619G>A / p.Ala129Thr | √ | | | | | | 31, 32 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *AANAT* | | c.-263G>C | | √ | √ | | | | 33 |
| | *BMAL (ARNTL2)* | rs922270 | g.24165C>T | √ | | | | | | 30 |
| | *BHLHE41* | MIM:612975 | c.1151C>G / p.Pro384Arg | | | | | √ | | 90 |
| | *BHLHE41* | | c.1151C>A / p.Pro384Gln | | | | | √ | √ | 92 |
| | *BHLHE41* | | c.1086C>T / p.Tyr362His | | | | | √ | √ | 92 |
| Adenosine | *ADA* | rs73598374 | c.22G>A / p.Asp8Asn | | | √ | √ | √ | √ | 53-55 |
| | *ADORA2A* | rs5751876 | c.1976T>C | | | √ | √ | √ | √ | 53, 117, 118 |
| Neurotrans-mitters | *GRIA3* | rs687577 | g.123445253A>C | | | | | √ | | 93 |
| | *COMT* | rs4680 | c.544G>A / p.Val158Met | √ | √ | √ | √ | √ | √ | 63, 64, 119 |
| Transporters | *SLC6A3 (DAT1)* | rs28363170 | VNTR | √ | √ | √ | √ | √ | √ | 59, 119 |
| | *SLC6A4 (5-HTT)* | rs687577 | 5-HTTLPR | | | | | √ | | 103 |
| Potassium channel | *ABCC9* | rs11046205 | g.102303C>T | | | | | √ | | 34 |
| | *ABCC9* | rs11046209 | g.97663T>A | | | | | √ | | 107 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Signaling pathways | *BDNF* | rs6265 | c.196G>A / p.Val66Met | | | √ | √ | | √ | 66, 67 |
| | *PRNP* | rs1799990 | c.385A>G / p.Met129Val | | | √ | | | | 72 |
| | *PAX8* | rs1823125 | g.114090412A>G | | | | | √ | | 107a |
| Immune response | *DQB1 *0602* | | | | | | | | √ | 134 |

Gene: NCBI gene symbol. NCBI SNP-ID number: National Center for Biotechnology Information (NCBI) single nucleotide polymorphism reference number. Base change: Nucleotide substitution at indicated position of coding DNA. Amino acid change: Amino acid substitution associated with base change. √: Possible contribution to phenotypic variation was investigated and reported.

**Legends to the Figures**

**Figure 1.** High *inter*-individual variation (left) and high *intra*-individual stability (right) in all-night EEG power spectra in NREM sleep in 32 baseline nights of 8 young men (S1 - S8). The largest inter-individual variation is observed in theta, alpha and sigma frequencies (~ 5-15 Hz). The spectra of all 4 baseline nights (BL1 - BL4) of one individual (S8) are virtually superimposable. Adapted and modified from Buckelmüller et al., *Neuroscience*, 2006.

**Figure 2.** Heritability of NREM sleep EEG is over 90 %. Panels show color-coded similarity indices of 8-16 Hz activity in monozygotic (left) and dizygotic (right) twin pairs. The similarity index in each twin pair was scaled between minimal (0 % similarity, white) and maximal (100 % similarity, dark orange). Black lines: derivation Fz, blue lines: derivation Cz, red lines: derivation: Pz (unipolar derivations referenced to averaged mastoid). Modified from De Gennaro et al., *Ann Neurol*, 2008.

**Figure 3.** The Val158Met polymorphism (rs4680) of the gene encoding catechol-O-methyltransferase (COMT) modulates EEG alpha activity in NREM sleep (all-night power spectra of stages 2-4). Black triangles at the bottom of the panels indicate frequency bins, which differ significantly between Val/Val (n = 10, black lines) and Met/Met (n = 12, red lines) genotypes ($p < 0.05$, unpaired, two-tailed t-tests). The frequency-specific effect of the genetic variation is robust against the effects of prolonged wakefulness and the stimulant modafinil. Data re-plotted from Bodenmann et al., *J Neurosci*, 2009.

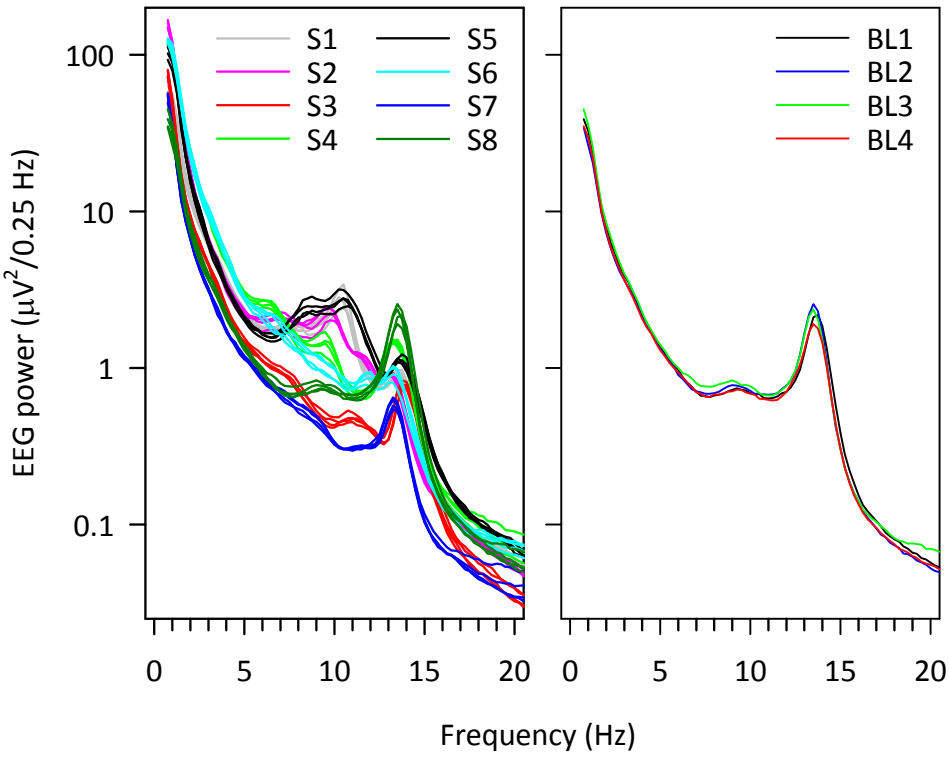
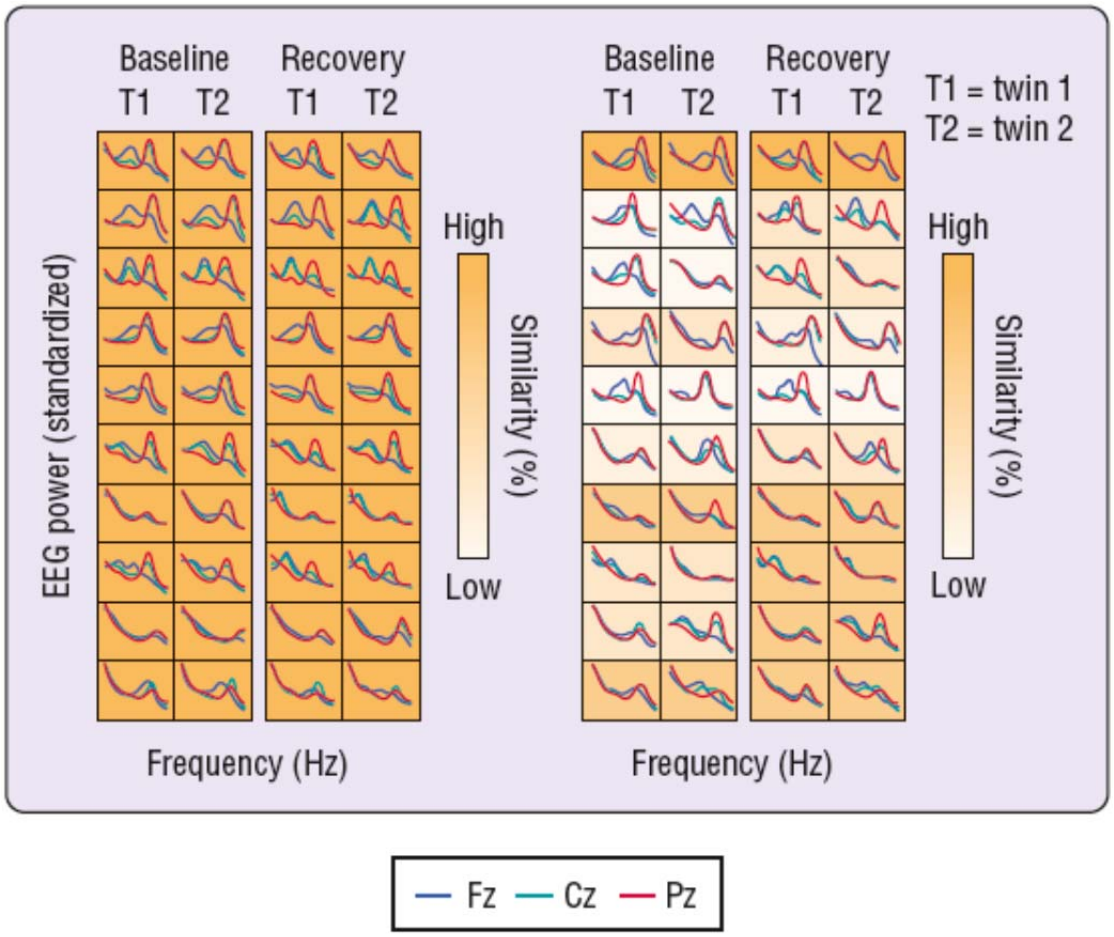
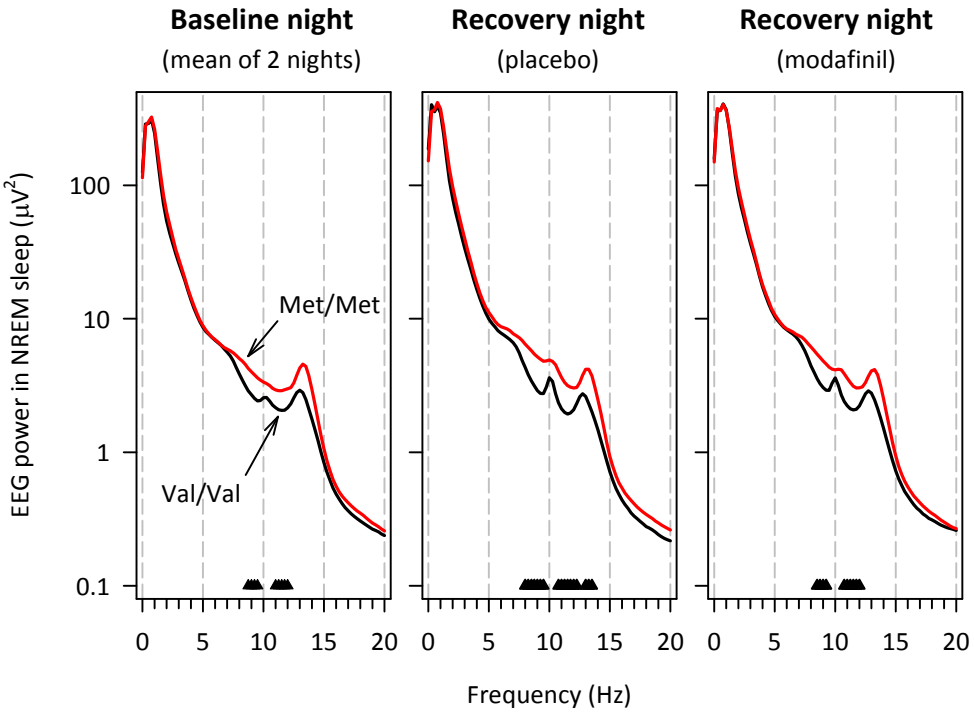

S1
S2
S3
S4
S5
S6
S7
S8
BL1
BL2
BL3
BL4
100
10
1
0.1
EEG power (μV²/0.25 Hz)
0
5
10
15
20
Frequency (Hz)

Figure 1


Baseline
Recovery
T1
T2
T1
T2
EEG power (standardized)
High
Similarity (%)
Low
Frequency (Hz)
Baseline
Recovery
T1
T2
T1
T2
T1 = twin 1
T2 = twin 2
High
Similarity (%)
Low
Frequency (Hz)
Fz
Cz
Pz


Baseline night
(mean of 2 nights)
Recovery night
(placebo)
Recovery night
(modafinil)
EEG power in NREM sleep (μV²)
100
10
1
0.1
Met/Met
Val/Val
0
5
10
15
20
Frequency (Hz)